P950032

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Michael Sabolinski, M.D.
Senior Vice President
Medical Affairs and Corporate Development
Organogenesis, Inc.
150 Dan Road
Canton, Massachusetts 02021

NOV 10 1998

Re: P950032
Apligraf™ (Graftskin)

Dear Dr. Sabolinski:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) wishes to notify you of an error in the device tradename cited in our May 22, 1998 approval order for PMA P950032. This letter which incorrectly identified the subject device of PMA P950032 as "Living Skin Equivalent (LSE) Graftskin", should have identified the device tradename as "Apligraf™ (Graftskin)". Please maintain this information with your approval order in insure proper documentation of this regulatory action. In addition, we apologize for any inconvenience this error my have caused.

If you have any questions about this corrective action, please contact me at 301 594-2186.

Sincerely yours,

Kathy Poneleit
Director, Premarket Approval Program
Office of Device Evaluation
Center for Devices and
Radiological Health

P950032

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Michael Sabolinski, M.D.
Senior Vice President
Medical Affairs and Corporate Development
Organogenesis, Incorporated
150 Dan Road
Canton, Massachusetts 02021

MAY 22 1998

Re: P950032
Living Skin Equivalent (LSE) Graftskin
Filed: October 4, 1995
Amended: December 1, 1995, January 16, April 1, May 22, August 6 and 8, September 18, November 1, 1996 and January 27, March 12, April 4, 25, and 30, May 12 and 21, August 29, October 6, 16, and 28, November 20, December 4, 5, 8, 9, 10, 12, 15, 16, 22, 23 and 24, 1997 and January 6, 8, 9, 13, 20, and January 23, 1998, February 10 and 11, March 16 and 25, April 8, 13 and 17, 1998

Dear Dr. Sabolinski:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Apligraf. This device is indicated for for use with standard therapeutic compression for the treatment of non-infected partial and full-thickness skin ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy.

We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution, and use of this device are restricted to prescription use in accordance with 21 CFR 801.109 within the meaning of section 520(e) of the Federal Food, Drug, and Cosmetic Act (the act) under the authority of section 515(d)(1)(B)(ii) of the act. FDA has also determined that to ensure the safe and effective use of the device that the device is further restricted within the meaning of section 520(e) under the authority of section 515(d)(1)(B)(ii) insofar as the sale, distribution, and use must not violate sections 502(q) and (r) of the act.

This approval is also subject to the following additional conditions:

1. Regarding the purity of the transferrin used in device manufacture, data documenting the viral inactivation properties of the processing procedures used by your supplier will be submitted to FDA immediately after receipt of these data by Organogenesis, Inc. from the supplier.

2. The significance of the karyology data observed on the keratinocyte and fibroblast cells used in product manufacturing needs to be further evaluated . Please submit within one month of an approval order for this product the following protocols:

    a. A protocol designed to determine the longevity of Apligraf cells on patients with venous ulcers.

    b. Protocols for evaluating the karyology, morphology and neoplastic potential of all keratinocyte and fibroblast cell lines that will be used in future commercial products. Such data should include evaluations at both the MWCB stage and a cell stage that is as close to cellular senescence as possible. These evaluations should not only quantitate the extent of chromosomal changes but also look for specific markers known to predict neoplastic transformation of keratinocyte cells. All such analyses should be performed in a manner consistent with the methods published in "Report of Ad Hoc Committee on Karyological Control of Human Cell Substrates," *J. of Biol. Standard.* 1979, 7, 397-404 or a justification should be supplied.

Expiration dating for this device has been established and approved at 5 days at 20-37°C when the device is maintained in its packaging container.

CDRH will notify the public of its decision to approve your PMA by making available a summary of the safety and effectiveness data upon which the approval is based. The information can be found on the FDA CDRH Internet HomePage located at http://www.fda.gov/cdrh/pmapage.html. Written requests for this information can also be made to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857. The written request should include the PMA number or docket number. Within 30 days from the date that this information is placed on the Internet, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

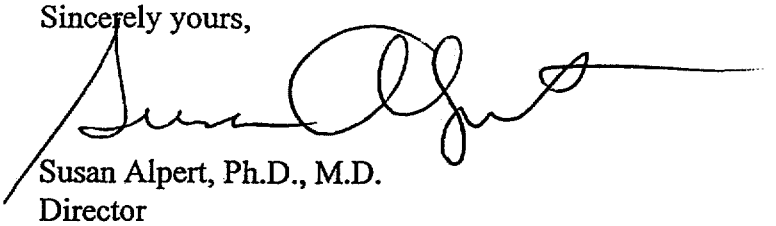
You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Charles N. Durfor, Ph.D. at (301) 594-3090.

Sincerely yours,



Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Issued: 3-4-98

CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but not the replacement of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

(a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

(b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mix-up of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3)Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984. This regulation was replaced by the reporting requirements of the Safe Medical Devices Act of 1990 which became effective July 31, 1996 and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to the FDA whenever they receive or otherwise become aware of information, from any source, that reasonably suggests that a device marketed by the manufacturer or importer:

(1)May have caused or contributed to a death or serious injury; or

(2)Has malfunctioned and such device or similar device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for a PMA, the manufacturer shall submit the appropriate reports required by the MDR Regulation within the time frames as identified in 21 CFR 803.10(c) using FDA Form 3500A, i.e., 30 days after becoming aware of a reportable death, serious injury, or malfunction as described in 21 CFR 803.50 and 21 CFR 803.52 and 5 days after becoming aware that a reportable MDR event requires remedial action to prevent an unreasonable risk of substantial harm to the public health. The manufacturer is responsible for submitting a baseline report on FDA Form 3417 for a device when the device model is first reported under 21 CFR 803.50. This baseline report is to include the PMA reference number. Any written report and its envelope is to be specifically identified, e.g., "Manufacturer Report," "5-Day Report," "Baseline Report," etc. Any written report is to be submitted to:

Food and Drug Administration
Center for Devices and Radiological Health
Medical Device Reporting
PO Box 3002
Rockville, Maryland 20847-3002

Copies of the MDR Regulation (FOD # 336&1336)and FDA publications entitled "An Overview of the Medical Device Reporting Regulation" (FOD # 509) and "Medical Device Reporting for Manufacturers" (FOD #987) are available on the CDRH WWW

Home Page. They are also available through CDRH's Fact-On-Demand (F-O-D) at 800-899-0381. Written requests for information can be made by sending a facsimile to CDRH's Division of Small Manufacturers Assistance (DSMA) at 301-443-8818.

9

P950032

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

DEVICE GENERIC NAME:	Graftskin
DEVICE TRADE NAME:	Apligraf™ (Graftskin)
APPLICANT:	Organogenesis Inc. 150 Dan Road Canton, MA 02021
PREMARKET APPROVAL APPLICATION (PMA):	P950032
DATE OF PANEL RECOMMENDATION:	January 29, 1998
DATE OF GMP INSPECTION:	April 8, 1996
DATE OF NOTICE OF APPROVAL OF APPLICATION:	May 22, 1998
EXPEDITED REVIEW:	Expedited processing was authorized on May 30, 1995, based on the potential of Apligraf™ to provide a clinically important advance over existing alternatives in the treatment of chronic venous insufficiency ulcers.

II. INTENDED USE / INDICATIONS

Apligraf is indicated for use with standard therapeutic compression for the treatment of non-infected partial and full-thickness skin ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy.

III. DEVICE DESCRIPTION

Apligraf is a viable, bi-layered, skin construct, which contains Type I bovine collagen, extracted and purified from bovine tendons and viable allogeneic human fibroblast and keratinocyte cells isolated from human infant foreskin. Apligraf™ consists of two primary layers. The upper "epidermal-like" layer, formed of living human keratinocytes, has a well

differentiated stratum corneum which has been shown in *in vitro* experiments to provide a natural barrier to topical infection and wound desiccation. In the supporting "dermis-like" layer of Apligraf™, the major cell type is the fibroblast. Apligraf™ fibroblasts produce many of the matrix proteins found in human dermis, such as collagen type IV, tenascin, decorin, hyaluronate, and fibronectin. In addition, collagen type IV, laminin, laminin 5, heparin sulfate, proteoglycan, and β_4 integrin are present at the dermal-epidermal junction. Apligraf also expresses many of the cytokines found in human skin including PDGF-A, PDGF-B, TGFα, TGFβ_1, TGFβ_3, ECGF, FGF-1, FGF-2, FGF-7, IGF-1, IGF-2, CSF, IL-1α, IL-6, IL-8 and IL-11. Other cells found in human skin, Langerhans cells, melanocytes, macrophages and lymphocytes as well as secondary structures such as blood vessels and hair follicles are not present in Apligraf.

Apligraf is supplied ready to use, in a plastic container/carrier and is intended for single use only. This container protects and supports the product and provides a supply of agarose gel nutrient medium to maintain cell viability until use. The carrier is sealed in a heavy gauge polyethylene bag containing a 10% CO_2/air atmosphere. Apligraf is kept in the sealed bag at 20-31°C until use. Apligraf is supplied as a circular disk 75 mm in diameter. The thickness of the product is 0.75 mm. The agarose shipping medium contains agarose, L-glutamine, hydrocortisone/bovine serum albumin, bovine insulin, human transferrin, triiodothyronine, ethanolamine, O-phosphorylethanolamine, adenine, selenious acid, DMEM powder, HAM's F-12 powder, sodium bicarbonate, calcium chloride and water for injection.

To maintain cell viability, the product is aseptically manufactured, but not terminally sterilized. Apligraf is shipped following a preliminary sterility test with a 48 hour incubation to determine the absence of microbial growth. Final (14 day incubation) sterility tests results are not available at the time of application.

Information concerning the following sections of this Summary of Safety and Effectiveness Data is included in the product labeling at the end of this document:

IV. CONTRAINDICATIONS

- Apligraf is contraindicated for use on clinically infected wounds.
- Apligraf is contraindicated in patients with known allergies to bovine collagen.
- Apligraf is contraindicated in patients with a known hypersensitivity to the components of the Apligraf agarose shipping medium.

The warnings and precautions can be found in the Apligraf labeling.

V. ALTERNATIVE PRACTICES AND PROCEDURES

Compression therapy is the standard of treatment for ulcers caused by venous disease. Surgical alternatives for venous ulcers include vein stripping, vein ligation and skin grafting.

VI. POTENTIAL ADVERSE EFFECTS

A total of 297 patients (161 Apligraf, 136 active control) were evaluated for safety in a clinical trial for the treatment of venous ulcers. Adverse events were recorded as mild, moderate, severe or life-threatening.

There were 1 life-threatening and 3 severe infections reported in the Apligraf™ group and none in the control arm. Of these, two severe infections were considered related to treatment: however one occurred one month after the last application of Apligraf and the other occurred following application on a pre-existing Pseudomonas infection.

All reported adverse events which occurred in the Apligraf cohort in the pivotal clinical study at an incidence of 1% or greater are listed in Table 1. The adverse events are listed in descending order according to frequency.

Table 1
Adverse Events Reported in Greater than 1.0% of Apligraf Patients

	Apligraf (n = 161)	Control (n= 136)
	Total	Total
Suspected Wound Infection[1] (study site)	47 (29.2%)	19 (14.0%)
Suspected Wound Infection[1] (non-study site)	16 (9.9%)	15 (11.0%)
Cellulitis[2] (study site)	13 (8.1%)	11 (8.1%)
Cellulitis[2] (non-study site)	12 (7.5%)	7 (5.1%)
Dermatitis (non-study site)	10 (6.2%)	10 (7.4%)
Exudate (study site)	9 (5.6%)	0 (0.0%)
Peripheral Edema	8 (5.0%)	7 (5.1%)
Pain (study site)	7 (4.3%)	7 (5.1%)
Death	6 (3.7%)	6 (4.4%)
Skin Ulcer (non-study site)	6 (3.7%)	5 (3.7%)
Pain (non-study site)	5 (3.1%)	4 (2.9%)
Pruritus (non-study site)	5 (3.1%)	2 (1.5%)
Skin Ulcer (study site)	5 (3.1%)	3 (2.2%)
Infection (non-wound)	4 (2.5%)	1 (0.7%)
Positive Wound Culture[3] (study site)	4 (2.5%)	3 (2.2%)
Rhinitis	4 (2.5%)	1 (0.7%)
Dermatitis (study site)	4 (2.5%)	2 (1.5%)
Pain (overall body)	3 (1.8%)	2 (1.5%)
Congestive Heart Failure	3 (1.8%)	0 (0.0%)
Accidental Injury (musculoskeletal)	3 (1.8%)	0 (0.0%)
Dyspnea	3 (1.8%)	1 (0.7%)
Pharyngitis	3 (1.8%)	0 (0.0%)
Rash (study site)	3 (1.8%)	2 (1.5%)
Accidental Injury (overall body)	2 (1.3%)	1 (0.7%)
Asthenia	2 (1.3%)	0 (0.0%)
Arrhythmia	2 (1.3%)	0 (0.0%)
Abscess (non-study site)	2 (1.3%)	0 (0.0%)
Arthralgia	2 (1.3%)	2 (1.5%)
Cough Increased	2 (1.3%)	0 (0.0%)
Rash (non-study site)	2 (1.3%)	5 (3.7%)
Erythema (study site)	2 (1.3%)	1 (0.7%)
Kidney Failure	2 (1.3%)	0 (0.0%)
Urinary Tract Infection	2 (1.3%)	5 (3.7%)

In the clinical trial the following definitions were used:

[1]*Suspected Wound infection:* a wound with at least some clinical signs and symptoms of infection such as increased exudate, odor, redness, swelling, heat, pain, tenderness to the touch and purulent discharge; quantitative culture was not required.

[2]*Cellulitis:* a non-suppurative inflammation of the subcutaneous tissues extending along connective tissue planes and across intercellular spaces; widespread swelling, redness and pain without definite localization.

[3]*Positive wound culture:* reported as an adverse event, but not reported as a wound infection.

VII. MARKETING HISTORY

Apligraf™ is approved for marketing in Canada and has been commercially available since August 12, 1997.

VIII. SUMMARY OF PRE-CLINICAL STUDIES

This section provides brief summaries of important preclinical tests performed on Apligraf followed by Table 2 which describes a number of non-clinical laboratory studies performed in the development and evaluation of Apligraf. Table 2 has been divided into studies chosen to evaluate the following categories: Development and Characterization, Immunology, Microbial and Toxicology studies. The studies reported here include a range of topics assessing safety, device attributes, practical aspects of device delivery, and potential clinical use issues.

Presence of Blood Group Antigens on Apligraf - DNA coding the Rh Factor was identified by PCR analysis of the cells used to make Apligraf for pivotal study. Weak, patchy staining of the B Blood Group antigen in the epidermal layer of this Apligraf was detected by immunohistochemical (IHC) analysis. No expression of the Rh antigen by Apligraf was observed in flow cytometry measurements.

Apligraf Compatibility with Antimicrobial Agents - In *in vitro* and *in vivo* histology studies, exposure to Dakin's solution, Mafenide Acetate, Scarlet Red Dressing, Tincoban, Zinc Sulfate, Povidone-iodine solution, or Chlorhexidine degraded the overall histology of Apligraf. Device exposure to Mafenide acetate, Polymixin/Nystatin or Dakin's Solution also reduced Apligraf cell viability.

Karyology analyses of keratinocyte cells used in device manufacture revealed a limited number of chromosomal abnormalities. These same cells were not neoplastic in *in vitro* and *in vivo* assays.

The fibroblast and keratinocyte cells, from which Apligraf is manufactured, are from human infant foreskin tissue. Products made from human tissue may contain infectious

agents. The risk that Apligraf will transmit a pathogenic agent is reduced by extensive testing. A comprehensive medical history of the mother was taken and blood of the mother was screened for HIV-1, HIV-2, HIV-p24, HTLV-1, HTLV-2, Hepatitis A, Hepatitis B surface, Hepatitis B core, Hepatitis B, Hepatitis C, Cytomegalovirus and Epstein Barr viruses. Additionally, human fibroblasts and keratinocytes used to form Apligraf are derived from cell banks which were tested for HIV-1, HIV-2, HIV-p24, HTLV-1, HTLV-2, Hepatitis B surface, Hepatitis C, Cytomegalovirus, Epstein-Barr virus, bacteria, fungi, yeast, mycoplasma, *in vitro* virus, *in vivo* virus, karyology, isoenzymes, virus by EM, Retrovirus by RT, Herpesvirus 6 and tumorigenicity. Product manufacture also includes reagents derived from animal materials including bovine pituitary extract. All animal-derived reagents are tested for viruses, retroviruses, bacteria, fungi, yeast, and mycoplasma before use and all bovine material is obtained from countries free of Bovine Spongiform Encephalopathy (BSE). To maintain cell viability, the product is aseptically manufactured, but not terminally sterilized. Apligraf is shipped following a preliminary sterility test with a 48 hour incubation to determine the absence of microbial growth. Final (14 day incubation) sterility tests results are not available at the time of application. The final product is also tested for morphology, cell viability, epidermal coverage, mycoplasma, and physical container integrity.

Table 2
Apligraf™ Pre-Clinical Studies

Development & Characterization Studies	
Study	**Results/Conclusions**
Cytokine and receptor analysis of Apligraf by RT-PCR	The cytokine and cytokine receptor mRNA profile of Apligraf and component cells were similar to human skin. However, hematopoietic cell derived cytokines (IL-2 and IL-4) were not detected. This is consistent with the use of pure HEP and HDF populations.
Apligraf: Determination of cell purity in HEP and HDF cell banks by flow cytometry	Results demonstrated that each HEP and HDF cell strain contained no detectable levels of professional APC (endothelial cells and Langerhan cells).
Determination of residual bovine serum proteins in Apligraf	NBCS in Apligraf G100 = 2.6 ± 0.07% total dry weight (4.5 mg per G100 unit).
Morphological development and maturation of Apligraf	Apligraf epidermis underwent a sequence of morphologic changes during development and maturation *in vitro* resulting in an organotypic skin culture with a morphology very similar to that of normal human skin. Changes in morphology parallel biochemical and functional events. Established morphological characteristics serve as device specifications.
Effect of Apligraf development on graft performance *in vivo*	100% graft take in mice; Apligraf epidermis remained throughout study; Basement membrane formed by day 15; TEM analysis confirmed the presence of the ultrastructural features of a differentiated epidermis.
Effect of Apligraf development on graft performance and barrier function formation *in vivo*	*In vitro* barrier function developed rapidly in mice between 14 and 20 days of culture. Apligraf (14d old) failed to integrate and persist on mice, while 16d old Apligraf persisted when grafted onto mice. The barrier function of the 16d old Apligraf grafted onto mice was slightly less than human skin. The barrier function of 20d old Apligraf grafted onto mice was comparable to human skin.
Immunology Studies	
Neutral allograft study[1]	HEPs and HDFs of Apligraf did not, but HUVECs did, stimulate T cell proliferation in a mixed lymphocyte reaction (MLR) assay.
Hu-SCID mouse study: Part I survival of Apligraf on Hu-SCID mice	Graft survival of Apligraf was significantly higher than human skin on hu-SCID mice ($p < 0.05$). After 28 days, 88% (n=7/8) of Apligraf grafts integrated and persisted on hu-SCID. In contrast, after 14 days, only 28% (n=2/7) of the human skin grafts persisted on hu-SCID mice. The survival of Apligraf and human skin on control SCID mice was not significantly different.
Hu-SCID mouse study: Part II survival of MHC class-II$^+$ Apligraf on Hu-SCID mice.	The persistence of IFN-γ treated Apligraf on hu-SCID mice (100% survival; n=9/9) was equivalent to the percent survival of untreated Apligraf on hu-SCID mice (100 % survival; n=10/10).
Regulation of T cell proliferation by keratinocyte derived soluble factors: part I	HEPs produce soluble factors that significantly inhibit the proliferation of anti-CD3 activated T cells.
Regulation of T cell proliferation by keratinocyte derived soluble factors: part II	HEPs produce soluble factors that significantly inhibit the proliferation of allogeneic T cells.

Table 2 (cont.)
Apligraf™ Pre-Clinical Studies

Immunology Studies (cont.)	
Study	**Results/Conclusions**
Identification of keratinocyte-derived T cell inhibitory factor	HEP inhibition of T cell proliferation did not require cell contact, was inducible in the presence of FBS, and could be partially blocked by addition of indomethecin or anti-TGF-β Mab. These results suggest that HEPs can regulate the response to antigen presented by other APC through the production of soluble factors.
Microbial Studies	
Can Apligraf act as a barrier to topical infection?	No evidence of bacterial penetration through the Apligraf was seen in a system where bacteria were seeded on the device supported on a membrane permeable to bacteria above sterile bacterial growth medium.
Toxicology Studies	
General Safety Test	Apligraf is non-toxic.
Primary Skin Irritation Study	No reactivity. Apligraf was scored as a non-irritant.
Kligman Maximization Study (sensitization assays)	No reactivity. Apligraf showed no primary irritancy response.
Tissue Culture-Agar Diffusion Test (cytotoxicity)	No reactivity. Apligraf met the requirements of the Agar Diffusion Test, USPXXII.
Systemic Injection Test	No reactivity. Apligraf is non-toxic.
Intracutaneous Test	No reactivity. Apligraf is non-toxic.
Hemolysis Test	No reactivity. Apligraf is non-hemolytic.
Subcutaneous Injection Test-Subchronic Toxicity	Apligraf caused a significant response when tested in albino rabbits. The protocol was designed for plastics or relatively inert materials. The validity of the test was compromised by the nature of Apligraf. Therefore, this test is not considered a valid measurement of toxicity for Apligraf.

APC: antigen presenting cell; HDF: human dermal fibroblast; HEP: human epidermal keratinocyte; RT-PCR: reverse transcriptase-polymerase chain reaction; NBCS: new born calf serum; TEM: transmission electron microscopy; IFN-γ: interferon-gamma; SCID: severe combined immunodeficient; MHC: major histocompatibility complex; HUVEC: human vascular endothelial cells; FBS: fetal bovine serum.

IX. SUMMARY OF THE RESULTS OF THE CLINICAL INVESTIGATION

The following is a summary of the large scale study designed to support approval, "Protocol 92-VSU-001, "Multi-Center Parallel Group Controlled Clinical Trial to Determine the Efficacy and Safety of Apligraf in the Treatment of Chronic Venous Insufficiency Leg Ulcers".

Study Design:
A prospective, randomized, controlled, multi-center, multi-specialty, unmasked study was conducted to evaluate the safety and effectiveness of Apligraf and compression therapy in comparison to an active treatment concurrent control of zinc paste gauze and compression therapy. The study population included consenting patients who were 18-89 years old, available for one year follow-up, with venous insufficiency confirmed by plethysmography (venous reflux < 20 sec.); associated with non-infected partial and / or full thickness skin loss ulcer (IAET Stage 2 or 3) of greater than one month duration and which had not adequately responded to conventional ulcer therapy. Patients were excluded for ankle brachial index < 0.65, severe rheumatoid arthritis, collagen vascular disease, pregnancy/lactation, cellulitis, osteomyelitis, ulcer with necrotic, avascular or bone/tendon/fascia exposed-bed, clinically significant wound healing impairment due to uncontrolled diabetes, or renal, hepatic, hematologic, neurologic or immune insufficiency or due to immunosuppressive agents such as corticosteroids (> 15 mg/day), radiation therapy or chemotherapy; or enrollment in studies within the past 30 days for investigational devices or within the past three months for investigational drugs related to wound healing.

Extremities with multiple ulcers were enrolled; however, only one ulcer per extremity was studied. Non-study ulcer care was not specifically defined. Study ulcer care was defined for the treatment (Apligraf and compression therapy) and control (zinc paste gauze and compression therapy), treatment groups in two phases:

1) Active Phase (0-8 weeks): All patients received: i) a non-adherent, ii) a non-occlusive and iii) a therapeutic compression dressing on day 0, mid-week during the first week (day 3-5), and at weeks 1-8. Control treated patients also received zinc impregnated gauze at each visit. All Apligraf patients received Apligraf on day 0. At the day 3-5 and weeks 1, 2 and 3 visits, if less than 50% Apligraf take was observed, then patients received an additional application of Apligraf. Patients were not allowed to receive more than 5 Apligraf applications total.

2) Maintenance Phase (8-52 weeks): Closed-ulcer extremities received non-specified elastic compression stockings. Open-ulcer extremities continued with dressing changes.

Study Endpoints:
The primary study endpoints were: 1) the incidence of 100% wound closure per unit time and 2) the overall incidence of 100% wound closure by 6 months. "Complete Wound Closure" was defined as full epithelialization of the wound with the absence of drainage. "Epithelialization" was defined as a thin layer of epithelium visible on the open wound surface. Secondary endpoint measurements included: the incidence of ulcer recurrence, duration of wound closure, immune responses against the human cellular and bovine device components and analyses of changes in ulcer: depth (IAET staging), erythema, edema, wound pain, fibrin, exudate, granulation tissue and overall assessment from baseline visit to the 6 month visit.

Listing of Study Centers and Patient Treatment Group Assignment:

The study enrollment is displayed below in Table 3.

Table 3
Patient enrollment by study site for the safety cohort*

Investigator & Center	Total # of Patients at a site
1. Gerit Mulder, Denver, CO	55
2. Oscar Alvarez, New Brunswick, NJ	50
3. Frank Maggiacomo, Providence, RI	41
4. Morton Altman, San Francisco, CA	24
5. Duyen Faria, Detroit, MI	22
6. Vincent Falanga, Miami, FL	19
7. James Snyder, Las Vegas, NV	18
8. Thomas Garland, Lawrenceville, NJ	17
9. George Mueller, San Diego, CA	16
10. Steven Bowman, Clearwater, FL	11
11. David Margolis, Philadelphia, PA	7
12. Thomas Schnitzer, Chicago, IL	6
13. Arnold Luterman, Mobile, AL	6
14. Marketa Limova, San Francisco, CA	4
15. John Hansbrough, San Diego, CA	1
TOTAL	**297**

Notes
*The product effectiveness dataset excluded the results from all patients treated at one clinical site, because FDA audit raised sufficient concerns about the reliability of the clinical records at this site. Consequently, the clinical outcome of these patients was excluded from study effectiveness analyses, but was included in all safety analyses. The dataset for product effectiveness included a total of 240 patients, i.e.,130 Apligraf and 110 Control patients.

Results:

Baseline Demographics:

The baseline demographics in both the Apligraf and Control arms were comparable for gender, race, age and ulcer area. Ulcer size was a little larger and longer in duration, (but not significantly) in the Apligraf treatment arm as displayed in Table 4.

Study Drop-outs:

The discontinuation rate for all patients prior to the 6 month evaluation was 76/291 (26%) and 105/291 (36%) at 12 months. Within the safety cohort, 59 Apligraf and 50 Control patients discontinued prior to 12 month visit.

Intent-to-treat Analyses of Ulcer Healing:

Apligraf use with standard therapeutic compression provided a statistically significant improvement in the incidence of ulcer closure per unit time for all patients enrolled in the effectiveness cohort when compared to control therapy in a Cox's Proportional Hazards Regression Analysis. The incidence of wound closure by 6 months was numerically superior, but not statistically significantly improved, in Apligraf-treated patients.

Chart 1

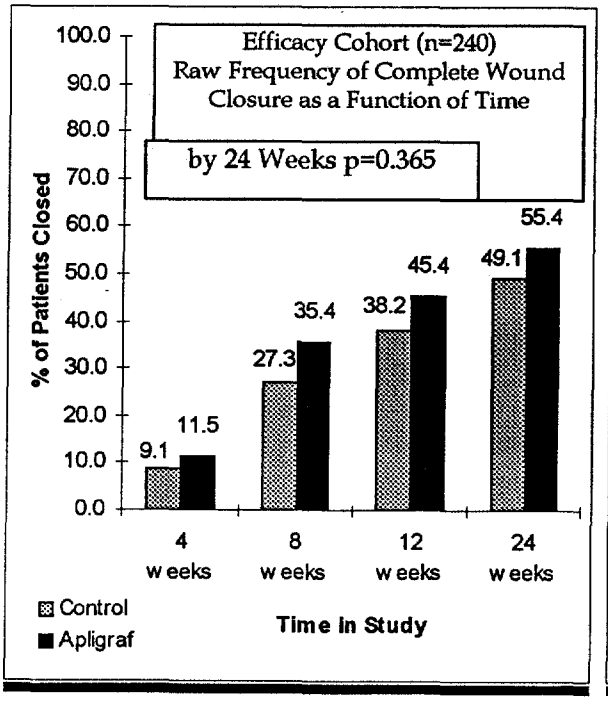
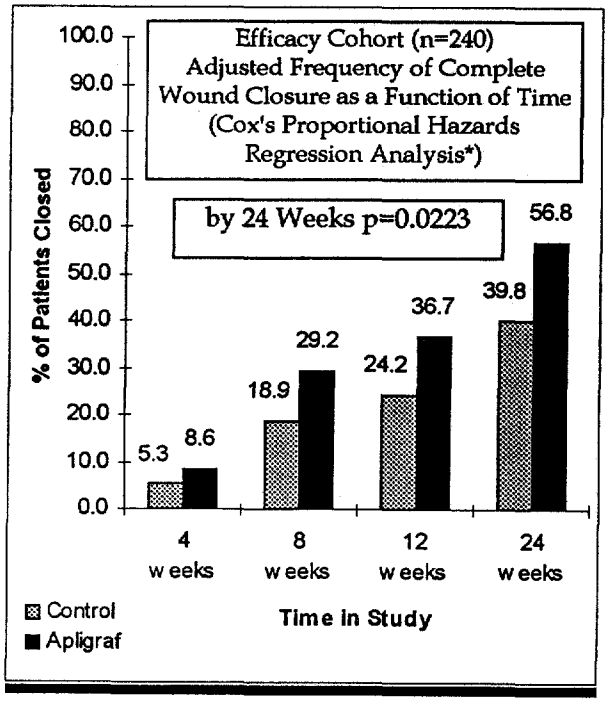


Chart 2



The incidence of wound closure at set visits up to 6 months presented as the raw data results (Chart 1) and the results after adjustment for pooled center, baseline ulcer duration and baseline area (Chart 2).

Incidence of Closure per Unit Time - In a Kaplan-Meier life table analysis, median times of 140 and 181 days were calculated for when 50% of the Apligraf and Control patients achieved wound closure, respectively, (p=0.3916). A Cox's Proportional Hazards Regression Analysis of these data determined that the covariables of pooled center, duration of ulcer and ulcer area had significant effects on the time to 100% wound closure for all patients. Adjusted median times to closure from this analysis were 99 and 184 days for Apligraf and Control patients, respectively.

Incidence of 100% Wound Closure - The overall closure rate was 55.4% (72/130) for Apligraf and 49.1% (54/110) for Control patients by 6 months (p= 0.365 by a Fisher's Exact 2-tailed test, Chart 1). In a logistic regression analysis of these data, the covariables of pooled center, baseline ulcer duration and baseline ulcer area were found to impact 100% wound closure for all patients. A logistic regression analysis, which adjusted for these factors, predicted that 58.8% of Apligraf and 44.0% of Control patients would achieve ulcer closure by six months (p = 0.0530). In a Cox's Regression Analysis, which accounted for the healing pattern over the six month timeline, closure rates of 56.8% and 39.8% by 24 weeks were predicted for Apligraf and Control patients, respectively, (p=0.0223, Chart 2).

Duration of Wound Closure - In this analysis once a patient achieved wound closure, the patient was judged a treatment success even if the duration of wound closure was short. The durability of complete wound closure was calculated from the first and last study days in which a Wound Closure case report form (CRF) was checked closed. In this analysis, the mean number of days for wound closure for patients who attained complete closure by 6 months and completed the study was 233 days for Apligraf patients and 219 days for Control patients. Similarly, the mean number of days of ulcer closure for all patients showed no significant differences for Apligraf (190 days) and Control (182 days) patients.

Correlation between photographs and Case Report Form records of wound closure was evaluated in a masked review with two evaluators of 437 study photographs composed of: 1) photographs of the baseline, time of first report of healing and the 6 month study visit for all 126 patients whose wound closure CRF was checked closed and 2) photographs at baseline, study week 8 and study month 6 of 20 Apligraf and 20 Control patients randomly selected from the 114 non-healing patients in the effectiveness cohort. These photographs, which represent 166 of the 240 patients in the effectiveness cohort, were randomly ordered to reduce bias or unmasking that might have resulted from a sequential ordering of the photographs. Results of this analysis revealed a good correlation between the data in Case Report Forms and the two reviewers with Kappa statistics ranging from 0.711 to 0.781.

Revised Effectiveness Cohort - Analysis of a revised dataset that selected only patients who met the precise study inclusion and exclusion criteria was performed. In this subset, the results of 32 patients were excluded from the intent-to-treat population because either: 1) they were over 85 years old, 2) their ulcers were not believed to be of non-venous etiology or 3) their ulcers were not of an appropriate size. The results of two additional Apligraf patients' ulcers were also switched from closed to open at 6 months after an FDA review of

clinical photographs. The improvement observed in Apligraf-treated over control treated patients in the incidence of ulcer closure per unit time remained statistically significant for this cohort (n=208).

Ulcer recurrence - At six months, the incidence of ulcer recurrence was 8.3% (6/72) for Apligraf- and 7.4% (4/54) for control-treated patients. The incidence of ulcer recurrence by 12 months was 18.1% (13/72) in the Apligraf group and 22.2% (12/54) in the control group.

Baseline status impact on wound closure - The impact of patient baseline status on wound closure was evaluated for the patients above and below the median values for ulcer duration and ulcer size as well as for baseline IAET Ulcer Stage, the presence of diabetes and a patient's Ankle Brachial Index. In these analyses, Apligraf use with standard compression provided statistically significant improvements in both: 1) the incidence of ulcer closure per unit time and 2) the incidence of ulcer closure by 6 months for patients with baseline ulcer durations greater than one year at baseline. The impact of baseline status impact on wound closure for different subgroups is displayed in Table 4.

Table 4
Pre-Treatment Status and Wound Closure
Effectiveness Cohort (n=240 patients)

	Pre-Treatment Status		Number and Percent of Wound Closure by 6 months	
Patient Condition	No. and (%) Apligraf Pts.	No. and (%) Control Pts.	Apligraf	Control
Total	130 Patients	110 Patients	72/130 (55.4%)	54/110 (49.1%)
Ulcer Duration				
≤ 1 year	58 (44.6%)	62 (56.3%)	38/58 (65.5%)	45/62 (72.6%)
> 1 year	72 (55.4%)	48 (43.6%)	34/72 (47.2%)	9/48 (18.8%)
***Ulcer Area**				
< 500 mm^2	65 (50.0%)	60 (54.5%)	45/65 (69.2%)	35/60 (58.3%)
> 500 mm^2	63 (48.5%)	50 (45.5%)	26/63 (41.3%)	19/50 (38.0%)
IAET Staging				
Stage II	63 (48.5%)	56 (50.9%)	34/63 (54.0%)	32/56 (57.1%)
Stage III	67 (51.5%)	54 (49.1%)	38/67 (56.7%)	22/54 (40.7%)
Diabetes				
Yes[1]	25 (19.2%)	11 (10.0%)	12/25 (48.0%)	4/11 (36.4%)
No	105 (80.8%)	99 (90.0%)	60/105 (57.1%)	50/99 (50.5%)
****Ankle Brachial Index data (ABI)**				
> 0.65 - < 0.8	9 (6.9%)	10 (9.1%)	4/9 (44.4%)	4/10 (40.0%)
>0.8 - <1.0	43 (33.1%)	50 (45.5%)	26/43 (60.5%)	27/50 (54.0%)
>1.0	75 (57.7%)	49 (44.5%)	40/75 (53.3%)	22/49 (44.9%)

*Baseline ulcer area missing for two patients in the Apligraf group
**ABI data is missing for 3 Apligraf and 1 control patient
[1] This category includes both insulin-dependent and non-insulin dependent diabetes patients, because the insulin-dependence of patients was not determined in this clinical trial

Secondary effectiveness endpoints - Changes in ulcer depth (IAET staging), erythema, edema, wound pain, fibrin, exudate, granulation tissue and overall assessment were assessed. Statistically significant differences between treatment groups were found for wound exudate at day 3-5 and week 2. The Apligraf group experienced earlier improvement in fibrin, while the Control group showed earlier improvement in wound exudate. While both treatment arms showed statistically significant improvement in all clinical parameters and patient overall assessments when comparing the baseline and 6 month visits, no statistically significant differences between treatment groups were observed at the 6 month visit.

Gender and Wound Closure - 36/70 (51%) of the men and 36/60 (60%) of the women in the Apligraf treatment group achieved wound closure by six months. In the Control group 19/52 (37%) of the men and 35/58 (60.8%) of the women in the Control treatment arm achieved wound closure by six months. The distribution of men (36.5%) and women (60.3%) attaining 100% wound closure in the Control arm was statistically significant by a Fisher's Exact 2-tail test (p=0.014).

Device Safety

Study Withdrawals:

13 patients withdrew from Study 92-VSU-001 due to adverse events or intercurrent illness. Per treatment arm the division was 5 Apligraf patients (1 male and 4 females) and 8 Control patients (4 males and 4 females).

Adverse events: Are displayed in section VI.

Suspected wound infection at the study ulcer: - 47/161 Apligraf (29.2%) and 19/136 (14.0%) Control patients had reports of localized suspected wound infections at the study site as defined by a wound with at least some clinical signs and symptoms of infection such as redness, swelling, heat, pain, tenderness to the touch and purulent discharge. The difference between treatment arms was significant (p=0.002) for all wound infections and non-significant (p=0.190) for wound infections judged to be device related. Overall there were 12/46 (26.1%) and 6/18 (33.3%) suspected wound infections judged as related to Apligraf and Control treatments, respectively. There were 1 life-threatening and 3 severe infections in the Apligraf group and none in the control arm. While the life threatening infection was judged as unrelated to device application, two of three severe infections were judged as Apligraf treatment-related. One Control (and no Apligraf) patient was hospitalized for infection at the study ulcer.

Because quantitative wound culture was not performed routinely in the study, the true incidence of wound infection associated with Apligraf use remains unknown. Diagnosis of wound infection may be complicated by the white or yellow appearance of Apligraf after it becomes hydrated with wound fluid.

Immune response:
In tests of patients' sera there were no observations of antibody responses against bovine type I collagen, bovine serum proteins or the Class I HLA antigens on human dermal fibroblasts and human epidermal cells. T-cell specific responses were not observed against bovine type I collagen, human fibroblasts or human keratinocytes. There was also no clinical evidence of Apligraf rejection by any patient.

X. CONCLUSIONS DRAWN FROM THE STUDY

This study provides reasonable assurance of the safety and effectiveness of Apligraf with standard therapeutic compression for the treatment of non-infected partial and full-thickness skin ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy. This study demonstrated that:

- Apligraf provides a statistically significant advantage in the incidence of wound closure per unit time when used with standard therapeutic compression for the treatment of non-infected partial and full-thickness skin ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy. The incidence of wound closure by 6 months was numerically superior, but not statistically significantly improved in patients treated with Apligraf.

- In the controlled clinical study conducted in patients with ulcers due to venous insufficiency of greater than one month in duration, suspected infection was reported more frequently in Apligraf-treated (29.2%) than control patients (14.0%). There were 1 life-threatening and 3 severe infections in the Apligraf group and none in the control arm.

- There were no observations of antibody responses against bovine type I collagen, bovine serum proteins or the Class I HLA antigens on human dermal fibroblasts, and human epidermal cells. T-cell specific responses were also not observed against bovine type I collagen, human fibroblasts or human keratinocytes.

XI. PANEL RECOMMENDATION

On January 29, 1998, the General and Plastic Surgery Devices Panel recommended approval without conditions of Organogenesis' PMA for Apligraf. In these discussions the Panel agreed that the definition of wound healing used in the pivotal study, (i.e., full epithelialization of the wound with the absence of drainage, where epithelialization was

defined as a thin layer of epithelium visible on the open wound surface) was consistent with the definition of a "healed" ulcer.

XII. CDRH ACTION

Expedited processing was authorized on May 30, 1995, based on the potential of Apligraf™ to provide a clinically important advance over existing alternatives in the treatment of chronic venous insufficiency ulcers.

Inspection of the sponsor's manufacturing facilities was completed on April 8, 1996 and was found to be in compliance with the device Good Manufacturing Practice regulations.

After the Panel meeting, FDA completed review of preclinical testing and product manufacturing issues. These issues involved assessing the purity and composition of device components and manufacturing reagents. In specific:

1) It was determined that the use of bovine pituitary extract (obtained from a BSE-free country) in the keratinocyte growth media should be identified in the device description.

2) The keratinocyte cells in this device were found to weakly express the B Blood Group antigen, but not the Rh antigen. Fibroblasts did not express either antigen. These results are consistent with the scientific literature on cultured skin products. Based on the results of studies with Apligraf, the extensive clinical use of cadaver skin and the scientific literature on cultured skin products, the weak expression of Blood Group antigens on the device was not believed to be clinically significant.

3) The purity of transferrin used in device manufacture was reviewed and determined to be safe. Submission of formal documentation about the methods of inactivating blood-borne viruses during preparation this plasma-derived reagent was requested as a condition of product approval.

4) Karyology analyses of keratinocyte cells used in device manufacture revealed a limited number of chromosomal abnormalities. These same cells were not neoplastic in *in vitro* and *in vivo* assays. As a condition, of approval the sponsor was requested to evaluate these findings with respect to the longevity of Apligraf cells on venous ulcer patients and the karyology, morphology and neoplastic potential of all keratinocyte and fibroblast cell lines used in the manufacture of future commercial products.

FDA issued an approval order on May 22, 1998.

APPROVAL SPECIFICATIONS

Directions for Use: See product labeling.

Postapproval Requirement and Restrictions:

1. Regarding the purity of the transferrin used in device manufacture, data documenting the viral inactivation properties of the processing procedures used by your supplier will be submitted to FDA immediately after receipt of these data by Organogenesis, Inc. from the supplier.

2. The significance of the karyology data observed on the keratinocyte and fibroblast cells used in product manufacture needs to be further evaluated. Please submit within one month of an approval order for this product the following protocols:

    a. A protocol designed to determine the longevity of Apligraf cells on patients with venous ulcers.

    b. Protocols for evaluating the karyology, morphology and neoplastic potential of all keratinocyte and fibroblast cell lines that will be used in future commercial products. Such data should include evaluations at both the MWCB stage and a cell stage that is as close to cellular senescence as possible. These evaluations should not only quantitate the extent of chromosomal changes, but also look for specific markers known to predict neoplastic transformation of keratinocyte cells. All such analyses should be performed in a manner consistent with the methods published in "Report of Ad Hoc Committee on Karyological Control of Human Cell Substrates," *J. of Biol. Standard.* 1979, 7, 397-404 or a justification should be supplied.

REFERENCE

[1] Theobald VA, Lauer JD, Kaplan FA, Baker KB, Rosenburg M. Neutral Allografts-Lack of Allogeneic Stimulation by Cultured Human Cells Expressing MHC Class I and Class II Antigens. Transplantation 1993;55:128-33.

Caution: **Federal Law restricts this device to sale by or on the order of a physician (or properly licensed practitioner).**

1. DEVICE DESCRIPTION

Apligraf is a viable, bi-layered, skin construct: the epidermal layer is formed by human keratinocytes and has a well-differentiated stratum corneum; the dermal layer is composed of human fibroblasts in a bovine Type I collagen lattice. While matrix proteins and cytokines found in human skin are present in Apligraf, Apligraf does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels or hair follicles.

Apligraf is manufactured under aseptic conditions from human neonatal male foreskin tissue. The fibroblast and keratinocyte cell banks which are the source of the cells from which Apligraf is derived are tested for human and animal viruses, retroviruses, bacteria, fungi, yeast, mycoplasma, karyology, isoenzymes and tumorigenicity. The final product is tested for morphology, cell viability, epidermal coverage, sterility, mycoplasma, and physical container integrity. Product manufacture also includes reagents derived from animal materials including bovine pituitary extract. All animal-derived reagents are tested for viruses, retroviruses, bacteria, fungi, yeast, and mycoplasma before use and all bovine material is obtained from countries free of Bovine Spongiform Encephalopathy (BSE).

2. INTENDED USE / INDICATIONS

Apligraf is indicated for use with standard therapeutic compression for the treatment of non-infected partial and full-thickness skin ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy.

3. CONTRAINDICATIONS

- Apligraf is contraindicated for use on clinically infected wounds.
- Apligraf is contraindicated in patients with known allergies to bovine collagen.
- Apligraf is contraindicated in patients with a known hypersensitivity to the components of the Apligraf agarose shipping medium (Section 8).

4. WARNINGS

Warning: DO NOT OPEN AND DO NOT USE Apligraf after the expiration date or if the pH is not within the acceptable range (6.8-7.7) as determined by the provided color chart. (Section 9).

Warning: Allergic reactions to the components in the Apligraf agarose shipping medium (Section 8) and bovine collagen, (a component of Apligraf), have been reported. Discontinue product use if a patient shows evidence of an immunologic reaction. Patients should notify their physician of any symptoms of an allergic

reaction. In studies with 361 patients, no allergic reactions to Apligraf were reported.

5. PRECAUTIONS

Caution: Do not use Apligraf if there is evidence of container damage or product contamination.

Caution: Apligraf should not be reused, frozen or sterilized after opening.

Caution: Apligraf should be kept in its tray on the shipping medium in the sealed bag under controlled temperature (20-31°C) until ready for use.

Caution: Apligraf should be handled using sterile technique and placed on a prepared wound bed within 5 minutes of opening the package.

Caution: Do not use cytotoxic agents, including Dakin's solution, Mafenide Acetate, Scarlet Red Dressing, Tincoban, Zinc Sulfate, Povidone-iodine solution, or Chlorhexidine with Apligraf. In *in vitro* and *in vivo* histology studies, exposure to these agents degraded Apligraf. Device exposure to Mafenide acetate, Polymixin/Nystatin or Dakin's Solution also reduced Apligraf cell viability.

Caution: Diagnosis of wound infection may be complicated by the white or yellow appearance of Apligraf after it becomes hydrated with wound fluid. Apligraf-treated wounds with respect to signs of suspected infection, including a change from baseline at the ulcer site for pain, edema, erythema, drainage, odor, warmth and/or unexplained fever, should be evaluated and treated according to standard practice for infection.

Caution: The persistence of Apligraf cells on the wound and the safety of this device in venous ulcer patients beyond one year has not been evaluated. In clinical studies with Apligraf there have been no reports of long term sequelae associated with Apligraf use.

Caution: The safety and the effectiveness of Apligraf have not been established for patients receiving greater than 5 device applications.

6. ADVERSE EVENTS

All reported adverse events which occurred in the Apligraf cohort in the pivotal clinical study at an incidence of 1% or greater are listed in Table 1. The adverse events are listed in descending order according to frequency. This table lists all adverse events reported in the study including those attributed and not attributed to treatment.

Table 1
Adverse Events Reported in Greater than 1.0% of Apligraf Patients

	Apligraf (n = 161)	Control (n= 136)
	Total	Total
Suspected Wound Infection[1] (study site)	47 (29.2%)	19 (14.0%)
Suspected Wound Infection[1] (non-study site)	16 (9.9%)	15 (11.0%)
Cellulitis[2] (study site)	13 (8.1%)	11 (8.1%)
Cellulitis[2] (non-study site)	12 (7.5%)	7 (5.1%)
Dermatitis (non-study site)	10 (6.2%)	10 (7.4%)
Exudate (study site)	9 (5.6%)	0 (0.0%)
Peripheral Edema	8 (5.0%)	7 (5.1%)
Pain (study site)	7 (4.3%)	7 (5.1%)
Death	6 (3.7%)	6 (4.4%)
Skin Ulcer (non-study site)	6 (3.7%)	5 (3.7%)
Pain (non-study site)	5 (3.1%)	4 (2.9%)
Pruritus (non-study site)	5 (3.1%)	2 (1.5%)
Skin Ulcer (study site)	5 (3.1%)	3 (2.2%)
Infection (non-wound)	4 (2.5%)	1 (0.7%)
Positive Wound Culture[3] (study site)	4 (2.5%)	3 (2.2%)
Rhinitis	4 (2.5%)	1 (0.7%)
Dermatitis (study site)	4 (2.5%)	2 (1.5%)
Pain (overall body)	3 (1.8%)	2 (1.5%)
Congestive Heart Failure	3 (1.8%)	0 (0.0%)
Accidental Injury (musculoskeletal)	3 (1.8%)	0 (0.0%)
Dyspnea	3 (1.8%)	1 (0.7%)
Pharyngitis	3 (1.8%)	0 (0.0%)
Rash (study site)	3 (1.8%)	2 (1.5%)
Accidental Injury (overall body)	2 (1.3%)	1 (0.7%)
Asthenia	2 (1.3%)	0 (0.0%)
Arrhythmia	2 (1.3%)	0 (0.0%)
Abscess (non-study site)	2 (1.3%)	0 (0.0%)
Arthralgia	2 (1.3%)	2 (1.5%)
Cough Increased	2 (1.3%)	0 (0.0%)
Rash (non-study site)	2 (1.3%)	5 (3.7%)
Erythema (study site)	2 (1.3%)	1 (0.7%)
Kidney Failure	2 (1.3%)	0 (0.0%)
Urinary Tract Infection	2 (1.3%)	5 (3.7%)

In the clinical trial the following definitions were used:

[1]*Suspected Wound infection:* a wound with at least some clinical signs and symptoms of infection such as increased exudate, odor, redness, swelling, heat, pain, tenderness to the touch and purulent discharge; quantitative culture was not required.

[2]*Cellulitis:* a non-suppurative inflammation of the subcutaneous tissues extending along connective tissue planes and across intercellular spaces; widespread swelling, redness and pain without definite localization.

[3]*Positive wound culture:* reported as an adverse event, but not reported as a wound infection.

Adverse events were recorded as mild, moderate, severe or life-threatening. There were 1 life-threatening and 3 severe infections reported in the Apligraf group and none in the control arm. Of the four events, two severe infections were considered related to treatment: however one occurred one month after the last application of Apligraf and the other occurred following application on a pre-existing Pseudomonas infection.

7. CLINICAL STUDIES

Study Design - A prospective, randomized, controlled, multi-center, multi-specialty, unmasked study was conducted to evaluate the safety and effectiveness of Apligraf and compression therapy in comparison to an active treatment concurrent control of zinc paste gauze and compression therapy. The study population included consenting patients who were 18-88 years old, available for one year follow-up, with venous insufficiency confirmed by plethysmography (venous reflux < 20 sec.); associated with non-infected partial and / or full thickness skin loss ulcer (IAET Stage 2 or 3) of greater than one month duration and which had not adequately responded to conventional ulcer therapy. Patients were excluded for ankle brachial index < 0.65, severe rheumatoid arthritis, collagen vascular disease, pregnancy/lactation, cellulitis, osteomyelitis, ulcer with necrotic, avascular or bone/tendon/fascia exposed-bed, clinically significant wound healing impairment due to uncontrolled diabetes, or renal, hepatic, hematologic, neurologic or immune insufficiency or due to immunosuppressive agents such as corticosteroids (> 15 mg/day), radiation therapy or chemotherapy; or enrollment in studies within the past 30 days for investigational devices or within the past three months for investigational drugs related to wound healing.

Extremities with multiple ulcers were enrolled; however, only one ulcer per extremity was studied. Non-study ulcer care was not specifically defined. Study ulcer care was defined for the treatment (Apligraf and compression therapy) and control (zinc paste gauze and compression therapy), treatment groups in two phases:

1) Active Phase (0-8 weeks): All patients received: i) a non-adherent, ii) a non-occlusive and iii) a therapeutic compression dressing on day 0, mid-week during the first week (day 3-5), and at weeks 1-8. Control treated patients also received zinc impregnated gauze at each

visit. All Apligraf patients received Apligraf on day 0. At the day 3-5 and weeks 1, 2 and 3 visits, if less than 50% Apligraf take was observed, then patients received an additional application of Apligraf. Patients were not allowed to receive more than 5 Apligraf applications total.

2) Maintenance Phase (8-52 weeks): Closed-ulcer extremities received non-specified elastic compression stockings. Open-ulcer extremities continued with dressing changes.

Wound closure was defined as 100% epithelialization without drainage and assessed by clinical observation at visits on day 0, day 3-5, weekly from weeks 1-8, months 3 and 6 after initial treatment application or until wound closure was achieved. Additional follow-up visits were 9 and 12 months after initial treatment.

Study Results

The incidence of wound closure at set visits up to 6 months is presented below as the raw data results (Chart 1) and the results after adjustment for pooled center, baseline ulcer duration and baseline area (Chart 2).

Chart 1

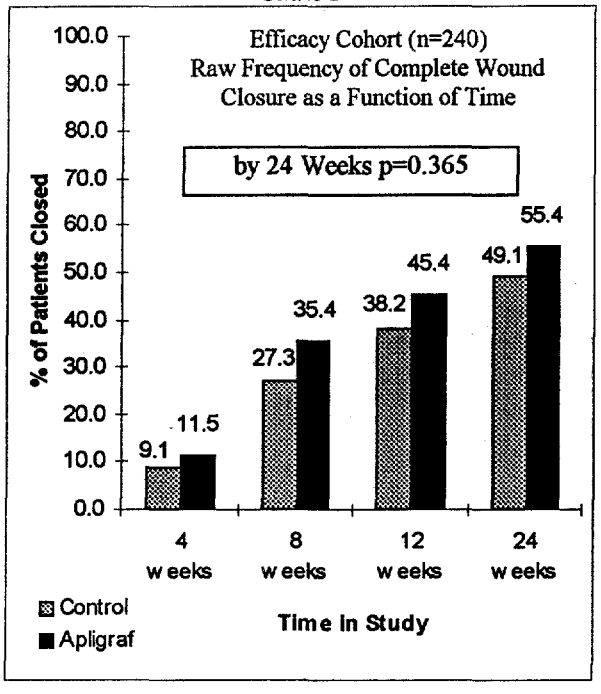
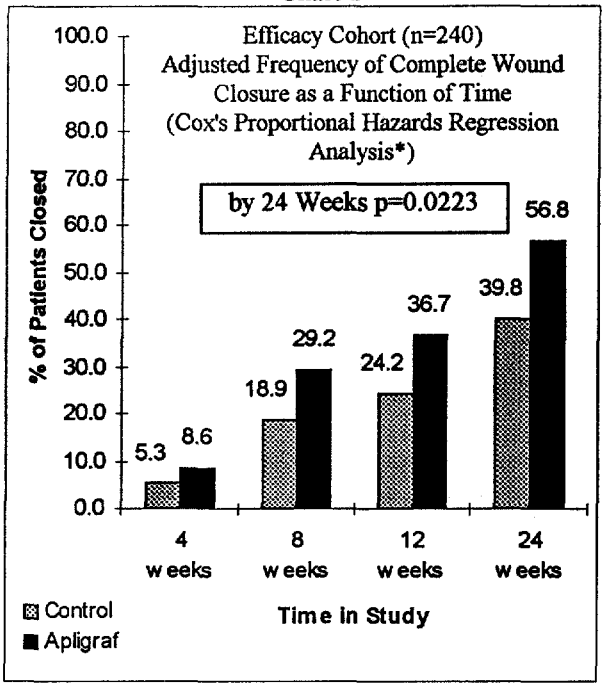


Chart 2



Ulcer recurrence
At six months, the incidence of ulcer recurrence was 8.3% (6/72) for Apligraf- and 7.4% (4/54) for control-treated patients. The incidence of ulcer recurrence by 12 months was 18.1% (13/72) in the Apligraf group and 22.2% (12/54) in the control group.

Suspected wound infection
In the effectiveness cohort, there were 33/130 (25.4%) Apligraf-treated and 15/110 (13.6%) control-treated ulcers with suspected wound infection. While the overall incidence of wound infection was higher in the Apligraf arm, the incidence of wound closure (Charts 1 and 2) was also higher for Apligraf -treated patients.

Baseline status impact on wound closure
The impact of patient baseline status on wound closure was evaluated for the patient populations above and below the median values for ulcer duration and ulcer size as well as for baseline IAET Ulcer Stage, the presence of diabetes and a patient's Ankle Brachial Index. The results of these analyses are displayed in Table 2.

Table 2
Pre-Treatment Status and Wound Closure
Effectiveness Cohort (n=240 patients)

	Pre-Treatment Status		Number and Percent of Wound Closure by 6 months	
Patient Condition	No. and (%) Apligraf Pts.	No. and (%) Control Pts.	Apligraf	Control
Total	130 Patients	110 Patients	72 (55.4%)	54 (49.1%)
Ulcer Duration				
≤ 1 year	58 (44.6%)	62 (56.3%)	38/58 (65.5%)	45/62 (72.6%)
> 1 year	72 (55.4%)	48 (43.6%)	34/72 (47.2%)	9/48 (18.8%)
***Ulcer Area**				
< 500 mm^2	65 (50.0%)	60 (54.5%)	45/65 (69.2%)	35/60 (58.3%)
> 500 mm^2	63 (48.5%)	50 (45.5%)	26/63 (41.3%)	19/50 (38.0%)
IAET Staging				
Stage II	63 (48.5%)	56 (50.9%)	34/63 (54.0%)	32/56 (57.1%)
Stage III	67 (51.5%)	54 (49.1%)	38/67 (56.7%)	22/54 (40.7%)
Diabetes				
Yes[1]	25 (19.2%)	11 (10.0%)	12/25 (48.0%)	4/11 (36.4%)
No	105 (80.8%)	99 (90.0%)	60/105 (57.1%)	50/99 (50.5%)
****Ankle Brachial Index data (ABI)**				
> 0.65 - < 0.8	9 (6.9%)	10 (9.1%)	4/9 (44.4%)	4/10 (40.0%)
>0.8 - <1.0	43 (33.1%)	50 (45.5%)	26/43 (60.5%)	27/50 (54.0%)
>1.0	75 (57.7%)	49 (44.5%)	40/75 (53.3%)	22/49 (44.9%)

*Baseline ulcer area missing for two patients in the Apligraf group
**ABI data is missing for 3 Apligraf and 1 control patient
[1] This category includes both insulin-dependent and non-insulin dependent diabetes patients, because the insulin-dependence of patients was not determined in this clinical trial

Secondary Endpoints
Clinical assessment (scale 1-4) of wound depth (IAET staging), erythema, edema, wound pain, fibrin, exudate, granulation tissue and overall assessment by changes in mean score and analysis of variance from baseline to the 6 month visit indicated no differences between treatment groups at 6 months.

Immune response:
In tests of patients' sera there were no observations of antibody responses against bovine type I collagen, bovine serum proteins or the Class I HLA antigens on human dermal fibroblasts, and human epidermal cells. T-cell specific responses were also not observed against bovine type I collagen, human fibroblasts or human keratinocytes. There was also no clinical evidence of Apligraf rejection by any patient.

8. HOW SUPPLIED

Apligraf is supplied sealed in a heavy gauge polyethylene bag with a 10% CO_2/air atmosphere and agarose nutrient medium, ready for single use. To maintain cell viability, Apligraf should be kept in the sealed bag at 20-31°C until use. Apligraf is supplied as a circular disk approximately 75 mm in diameter and 0.75 mm thick. The agarose shipping medium contains agarose, L-glutamine, hydrocortisone/bovine serum albumin, bovine insulin, human transferrin, triiodothyronine, ethanolamine, O-phosphorylethanolamine, adenine, selenious acid, DMEM powder, HAM's F-12 powder, sodium bicarbonate, calcium chloride and water for injection.

To maintain cell viability, the product is aseptically manufactured, but not terminally sterilized. Apligraf is shipped following a preliminary sterility test with a 48 hour incubation to determine the absence of microbial growth. Final (14 day incubation) sterility tests results are not available at the time of application.

9. DIRECTIONS FOR USE

Apligraf is indicated for use with standard therapeutic compression for the treatment of non-infected partial and full-thickness skin ulcers due to venous insufficiency of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy. Apligraf consists of living cells which must be kept sealed in its nutrient medium and 10% CO_2/air atmosphere under controlled temperature (20-31° C) and used within 5 minutes of opening.

Preparation of the Venous Ulcer Wound Bed Prior to Apligraf Application

1. **Wound Infection:**
   Apligraf should not be applied over infected or deteriorating wounds until the underlying condition has been resolved.

2. **Bacterial containment:**
   Antimicrobial agents may be used during the week prior to Apligraf application to reduce the risk of infection. Dakin's solution, Mafenide Acetate, Scarlet Red Dressing, Tincoban, Zinc Sulfate, Povidone-iodine solution, and Chlorhexidine have been determined to be cytotoxic to Apligraf.

3. **Wound bed preparation:**
   Apligraf should be applied to a clean, debrided wound after thoroughly irrigating the wound with a non-cytotoxic solution. Oozing or bleeding resulting from debridement should be stopped through the use of gentle pressure. Previous ulcer treatments other than standard therapeutic compression should be discontinued.

4. **Control of Heavy Exudation:**
   Heavy exudation may displace Apligraf and reduce adherence. Exudation should be minimized by appropriate clinical treatment. If exudation persists, Apligraf should be made permeable to exudate by perforating the Apligraf to allow for drainage.

Suggested Technique for the Application of Apligraf to the Wound

1. Check expiration date. If expired, do not open or use.
2. Check product pH. If not 6.8-7.7 by the provided color pH chart, do not open or use.
3. Prepare a sterile field and atraumatic instruments: forceps.
4. Cut open the sealed polyethylene bag and transfer the plastic tray to the sterile field with aseptic technique.
5. Lift off the tray lid and note epidermal and dermal layer orientation: Apligraf is packaged with the epidermal (dull, mattte finish) layer facing up and the dermal (glossy) layer facing down.
6. Using the sterile atraumatic instrument, gently dislodge approximately 0.5 inch of Apligraf away from the wall of the tray.
7. With sterile gloved hands, insert one index finger under the released section of Apligraf. Use the other index finger to grasp the Apligraf in a second spot along the edge of the device. Holding the Apligraf in two places lift the entire Apligraf out of the tray using a smooth, even motion. This easy motion should prevent Apligraf from bending and folding

over onto itself. To minimize Apligraf damage: avoid Apligraf contact with foreign bodies and minimize handling Apligraf except by its margins.

8. Do not allow Apligraf to fold or wrinkle on itself. If excessive folding occurs, Apligraf can be floated (epidermal surface up) onto warm sterile saline solution in a sterile tray.

9. Apligraf should be placed such that the dermal layer (the glossy layer closest to the medium) is in direct contact with the wound surface. Trim Apligraf so as to cover the wound bed with 1/8 -1/4" margins.

10. Secure Apligraf with a three layer dressing so as to assure contact to wound bed:

    - Apply a non-adherent dressing over the ulcer and Apligraf, extending 0.5 inch beyond the ulcer perimeter and inflamed skin margins.
    - Apply a non-occlusive dressing such as fine mesh gauze. This may be folded or rolled as a bolster.
    - Apply a self adherent elastic wrap from metatarsals to tibial plateau so that therapeutic compression is applied to the ulcer site.

Frequency of Dressing Changes and Apligraf Applications

1. The wound should be inspected and the dressing changed at least once a week during the immediate post application period. More frequent changes may be required on highly exudative wounds.
2. Additional applications of Apligraf may be necessary. Prior to additional applications, non-adherent remnants of Apligraf should be gently removed. Healing tissue or adherent Apligraf should not be disrupted. The wound bed should be cleansed with a non-cytotoxic solution prior to additional applications of Apligraf. Additional applications of Apligraf should not be applied over areas where Apligraf is adherent.
3. The wound site should continue to be dressed with a non-adherent dressing, pressure bolster and elastic overwrap as described above.
4. Upon complete wound closure, patients should be continued with compression therapy such as support stockings.
5. The safety and the effectiveness of Apligraf have not been established for patients receiving greater than 5 device applications.

10. PATIENT'S MANUAL

A brochure will be made available to:
1. Provide basic information about chronic wounds.
2. Address standard patient care while receiving Apligraf treatment
3. Educate patients on Apligraf-related healing process.

11. PEEL-OFF LABEL

Remove the peel-off label from the lower right corner of the Apligraf package label and place it in the patient's chart. This label bears a unique lot number and expiration date of the Apligraf.

Organogenesis Inc., APLIGRAF (Graftskin)
Essential Prescribing Information
Numbers in parentheses ( ) refer to sections in the main part of the product labeling

Device Description
Apligraf is a bi-layered viable skin construct manufactured using neonatal foreskin keratinocytes and fibroblasts with bovine Type I collagen. **(1)**

Intended Use/Indications
Apligraf is indicated for use with standard therapeutic compression in the treatment of uninfected partial and/or full-thickness skin loss ulcers due to venous insufficiency and of greater than 1 month duration and which have not adequately responded to conventional ulcer therapy. **(2)**

Contraindications
Apligraf is contraindicated for use on clinically infected wounds and in patients with known allergies to bovine collagen or hypersensitivity to the components of the shipping medium.**(3, 4, 5, 8)**

Warnings and Precautions
If the expiration date or product pH is not within the acceptable range (6.8-7.7) DO NOT OPEN AND DO NOT USE the product. A clinical determination of wound infection should be made based on all of the signs and symptoms of infection. **(4, 5)**

Adverse Events
In the controlled clinical study conducted in patients with ulcers due to venous insufficiency of greater than one month in duration, suspected infection was reported more frequently in Apligraf-treated (29.2%) than control patients (14.0%). There were 1 life-threatening and 3 severe infections in the Apligraf group and none in the control arm. Of these, two severe infections were considered related to treatment: however one occurred one month after the last application of Apligraf and the other occurred following application on a pre-existing Pseudomonas infection.

While the overall incidence of wound infection was higher in the Apligraf arm, the incidence of wound closure was 72/130 (55.4%) and 54/110 (49.1%) for Apligraf and Control treated patients, respectively. **(6)**

Maintaining Device Effectiveness
Apligraf has been processed under aseptic conditions and should be handled observing sterile technique. It should be kept in its tray on the medium in the sealed bag under controlled temperature (20-31°C) until ready for use. Apligraf should be placed on the wound bed within 5 minutes of opening the package. Handling before application to the wound site should be minimal. If there is any question that Apligraf may be contaminated or compromised, it should not be used. Apligraf should not be used beyond the listed expiration date.(9)

Use in Specific Populations
The safety and effectiveness of Apligraf has not been established in pregnant women, acute wounds, burns and ulcers caused by diabetic neuropathy or pressure.

Patient Counseling Information
Patients should be counseled regarding the importance of complying with compression therapy or other treatment which may be prescribed in conjunction with Apligraf.

How Supplied
Apligraf is supplied sealed in a heavy gauge polyethylene bag with a 10% CO_2/air atmosphere and agarose nutrient medium, ready for single use. To maintain cell viability, Apligraf should be kept in the sealed bag at 20-31°C until use. Apligraf is supplied as a circular disk approximately 75 mm in diameter and 0.75 mm thick.